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# Glial cell line-derived neurotrophic factor concentration dependently improves disability and motor activity in MPTP-treated common marmosets

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**Abstract**

Glial cell line-derived neurotrophic factor (GDNF) has previously reduced motor deficits and preserved nigral dopamine neurones in rhesus monkeys with a unilateral MPTP-induced lesion of substantia nigra. We now report on the ability of GDNF to reverse motor deficits induced by parenteral administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to common marmosets resulting in bilateral degeneration of the nigrostriatal pathway. Prior to GDNF administration, all MPTP-treated animals showed akinesia or bradykinesia, rigidity, postural instability and tremor. Intraventricular injection of GDNF (10, 100 or 500 μg) at 9 and 13 weeks post MPTP treatment resulted in a concentration dependent improvement in locomotor activity and motor disability which became significant after administration of 100 and 500 μg of GDNF. The most prominent improvements were in alertness, checking movements, and posture. It is concluded that intraventricular GDNF administration improves bilateral Parkinsonian motor disability following MPTP treatment and this may reflect an action of GDNF on remaining nigral dopaminergic neurones. 

*Keywords:* Dopamine; GDNF (glial cell line-derived neurotrophic factor); Parkinson's disease; MPTP primate; Motor activity

## 1. Introduction

Current therapeutic approaches to Parkinson's disease rely primarily on dopamine replacement therapy with L-dihydroxyphenylalanine (L-DOPA), or agonist-mediated dopamine receptor stimulation. However, such symptomatic treatments have no effect on disease progression and seem not to reverse the neurodegenerative changes occurring in the substantia nigra. Neurotrophic factors may provide one means of achieving neuroprotection or neurorestoration in Parkinson's disease. Indeed, there has been a number of previous attempts to reverse nigral cell degeneration in both 6-hydroxydopamine-treated and MPTP-treated primates using basic fibroblast growth factor (bFGF), epidermal growth factor (EGF) and brain derived neurotrophic factor (BDNF) (Du et al., 1995; Olson, 1997; Pearce et al., 1996; Pearce, 1999; Ventrella, 1993). However, none of these have been found to be clinically effective due to a lack of selectivity for dopaminergic neurones and/or difficulties in achieving intraventricular or focal administration into brain (Connor and Dragunow, 1998).

Glial cell line-derived neurotrophic factor (GDNF) is a novel protein purified and cloned on the basis of its marked ability to promote dopaminergic neuronal function (Lin et al., 1993). More recently, intracerebral administration of GDNF was found to exert both a neuroprotective and neurorestorative effect on the nigrostriatal pathway in MPTP-treated mice. GDNF improved the number of midbrain tyrosine hydroxylase (TH) positive dopamine neurones, and increased striatal nerve terminal density and dopamine levels (Tomac et al., 1995; Cheng et al., 1998; Date et al., 1998). Intrastriatal administration of GDNF (0.1–10 μg) similarly protected against 6-hydroxydopamine lesioning of the nigrostriatal pathway as judged by a

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reduction in amphetamine-induced rotation, preservation of TH-immunoreactive fibres in the striatum and protection of TH-immunoreactive neurones in the substantia nigra pars compacta (Shults et al., 1996). Similarly, supranigral injections of 10 μg GDNF at 4-day intervals following bilateral striatal injection of 6-hydroxydopamine caused a long lasting improvement in nigral dopamine cell number, although it had no effect on motor behaviour or the reduction in striatal dopamine terminal density (Winkler et al., 1996).

In primates, there has only been limited study of GDNF actions in models of Parkinson's disease. In rhesus monkeys with a unilateral lesion of the substantia nigra induced by intracarotid MPTP injection, the intracerebral (intranigral, intracaudate or intracerebroventricular) administration of GDNF ipsilateral to the side of the MPTP lesion, dose dependently improved bradykinesia, rigidity, and postural instability (Gash et al., 1996; Zhang et al., 1997; Miyoshi et al., 1997). On the lesioned side, GDNF treatment increased dopamine levels in the substantia nigra, ventral tegmental area, and globus pallidus but not in the striatum. However, interestingly, midbrain dopamine neurones showed a bilateral increase in TH-immunoreactive and cell size following GDNF administration making it difficult to assess the source of the motor improvement. Since there has not previously been any study of the effects of GDNF in bilaterally MPTP-lesioned primates, we now report on the ability of intracerebroventricularly administered GDNF to reverse motor deficits in common marmosets with a bilateral lesion of substantia nigra induced by subcutaneous MPTP administration. In this study, we confirm and extend the previous findings in rhesus monkeys showing a concentration dependent effect of GDNF on both MPTP-induced motor disability and locomotor activity.

## 2. Materials and methods

### 2.1. Animals

Adult common marmosets (*Callithrix jacchus*; n = 16) weighing 270–440 g at the beginning of the experiment, were housed under a 12-h light/dark cycle with free access to Mazuri Primate Expanded Diet (Special Diet Services) and drinking water. They were fed once daily with a variety of fresh fruits including bananas, apples, and oranges plus weekly vitamin D3 supplements and high protein Mazuri marmoset jelly. Animals were housed either in pairs or individually at a temperature of 25 ± 1° and 50% relative humidity. Following MPTP treatment, animals were hand fed on a cocktail of Mazuri marmoset jelly, dried milk, and pureed fruit until the MPTP-induced decline in body weight had been reversed. Experiments were carried out in accordance with the "Animals (Scientific Procedures) Act 1986".

### 2.2. MPTP treatment

Common marmosets were treated once daily with MPTP hydrochloride dissolved in sterile 0.9% saline (2 mg/kg s.c. into a small shaved area on the lower back) for 5 consecutive days such that a cumulative dose of 10 mg/kg was administered. After the last dose of MPTP, all animals showed marked motor deficits comprising, akinesia, hunched posture, rigidity, loss of vocalisation, and the emergence of postural action or body tremor. During the first 3 to 4 weeks following MPTP treatment, animals received daily hand feeding and their body weight was closely monitored, stabilising at no less than 91% of their original body weight. Five weeks after the last MPTP treatment, motor function had stabilised at a point where animals were able to groom, feed, and drink.

### 2.3. GDNF injection

Nine weeks after the first administration of MPTP, the animals were anaesthetised using Saffan 18 mg/kg i.m. (alphaxalone 0.9% and alphadolone acetate 0.3%; Pitman-Moore, UK) and had their head hair trimmed close with electric clippers. Synulox (0.1 ml s.c. clavulanic acid 35 mg and amoxacylin 140 mg; Beecham Animal Health) was administered as a prophylactic antibiotic. The animals were randomly assigned to four groups ($n = 4$), each group receiving varying concentrations of human recombinant methionine GDNF ranging from 10, 100, and 500 μg/5 μl (Amgen, Thousand Oaks, CA, USA) or 0.9% sterile saline. The animals were placed in a Kopf stereotaxic frame and after the conjunctivae were smeared with Aureomycin ophthalmic ointment (chlortetracycline hydrochloride 10 mg/g; Cyanamid, UK, Animal Health Division), the scalp was opened and at the stereotaxic coordinates over the injection site, a 2-mm diameter burr hole was drilled into the skull. A 26-gauge blunt needle tip attached to a 10-μl Hamilton microsyringe was inserted into the left lateral ventricle at 7.5 mm anterior to zero plane, 1.5 mm lateral and ventrally, 13 mm superior to the zero plane according to the stereotaxic atlas of common marmoset brain (Stephan et al., 1980). GDNF (10, 100 or 500 μg) or saline was injected at a rate of 1 μl/min and the needle was left in place for an additional 10 min before being withdrawn. Four weeks after the first GDNF or vehicle injection, animals were anaesthetised as above and the infusion procedure was repeated in the same manner. Animals were killed 2 weeks following the final GDNF administration under deep pentobarbitone (Sagatal) anaesthesia.

### 2.4. Disability scoring

The following observer rating scale was used to assess motor disability each day at 1400: alertness (normal = 0, reduced = 1, slow = 2, absent = 3); checking movements (normal = 0, reduced = 1, absent = 2); eyes (normal = 0,

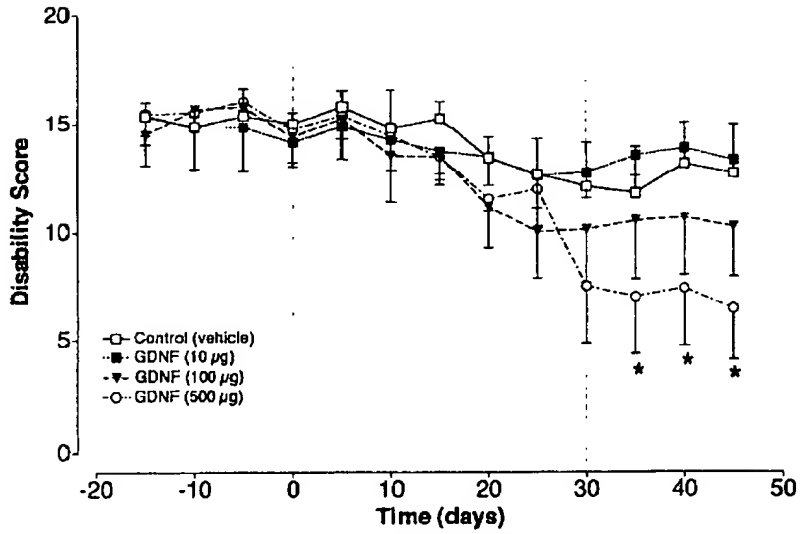


Fig. 1. The effect of GDNF treatment on basal disability score of the MPTP treated common marmosets. Dotted lines represent the time of surgery when vehicle or GDNF (10, 100 or 500 μg) were administered. Only after the second administration of 500 μg GDNF was there a statistically significant reduction in the disability score. Each data point represent mean ± S.E.M. of 5 days observation for each animal groups ($n = 4$). $^{*}P < 0.05$, ANOVA followed by Dunnett's test.

abnormal = 1); posture (normal = 0, slightly flexed = 1, moderately flexed = 2, very flexed = 3, head low = 4); balance (normal = 0, unstable = 1, falling = 2); motility (normal = 0, mild slowing = 1, moderate slowing = 1, distinct slowing = 3, akinesia = 4); vocalisation (normal = 0, reduced = 1, absent = 2); tremor (absent = 0, present = 1); fur condition (normal = 0, ungroomed = 1); rigidity (absent = 0, present = 1). The maximal possible disability score was 21. All animals were assessed in their home cages on a daily basis. The results from 5 consecutive days were then pooled to give a weighted score over each 5-day period.

### 2.5. Locomotor activity

Once weekly commencing on the 5th week following MPTP treatment, locomotor activity was measured in metal cages (50 × 60 × 70 cm) with perspex doors (50 × 70 cm) equipped with eight horizontal infrared beams and photocells. Two of the eight beams were above and parallel and two were above and perpendicular to each of the two perches. The number of light beam interruptions was accumulated in 10-m intervals and recorded for a total of 6 h using an Intel-based computer running Windows 3.1 operating system.

### 2.6. Statistical analysis

All data are presented as mean ± S.E.M. of absolute disability scores or locomotor counts. Disability and locomotor activity data following injections of vehicle or different concentrations of GDNF were initially analysed for normality of distribution. The Gaussian distribution of data was assessed using a modification of Kolmogorov-Smirnov (KS), using Prism software on a Macintosh computer. If samples followed the Gaussian distribution exactly, the KS distance would be zero. Analysis using this technique showed that all KS distances in each set of data were close to zero ranging from 0.145 to 0.373, thus passing the normality test. On the basis of this, changes in disability or locomotor activity were analysed by a repeated measure analysis of variance (ANOVA) where between subjects factor were the GDNF/vehicle treatments and within subjects factor were the time dependent mean disability scores. If significant statistical differences were detected between different factors, a post-hoc comparison of means for each

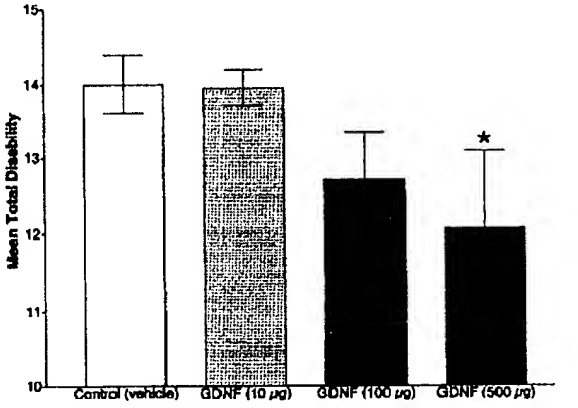


Fig. 2. Mean total disability scores for animals receiving vehicle or 10 to 500 μg GDNF. Each histogram represents pooled data for the disability scores of the animals following two administrations of vehicle or GDNF during the entire duration of the experiment. Error bars represent ± S.E.M. ($n = 4$) $^{*}P < 0.05$, ANOVA followed by Dunnett's test.

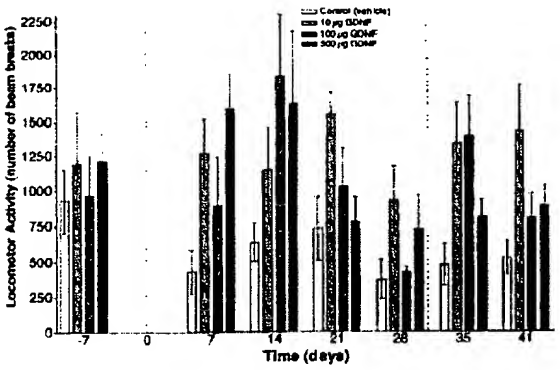


Fig. 3. The effect of GDNF treatment on the basal locomotor activity of the MPTP treated common marmosets. Dotted lines represent the time of each surgery. Each histogram represents mean ± S.E.M. ($n = 4$) of the total number of beam breaks over 6 h.

treatment time was performed between vehicle-treated and GDNF-treated groups using Dunnett's multiple comparison test.

## 3. Results

### 3.1. The effects of GDNF administration on motor disability

At the time of study, all animals exhibited rigidity, a loss of vocalisation, diminished blinking, incoordination and a course action tremor. There was no difference in the basal disability between groups prior to GDNF treatment. Following GDNF administration, we observed an overall difference between the effects of treatment ($P = 0.003$) and time ($P = 0.0001$) on motor disability scores (two-way ANOVA). In animals receiving vehicle or 10 μg GDNF, no significant differences in disability scores were present following the first or the second injections compared to the basal disability score ($P > 0.05$ ANOVA with repeated measures followed by Dunnett's multiple comparison test). The animals receiving 100 and 500 μg GDNF however displayed a time dependent trend towards reduced motor disability, peaking at 28 to 30 days after the first GDNF administration (Fig. 1). Only in animals receiving 500 μg GDNF was there a statistically significant ($P < 0.05$) improvement in the motor disability (Fig. 2). These improvements were most prominent with respect to alertness, checking movements, and posture (result not shown). Following the second administration of GDNF, no further improvements in motor disability were observed.

### 3.2. The effects of GDNF on locomotor activity

Prior to vehicle or GDNF administration, all animals displayed marked bradykinesia showing mean locomotor counts over 6 h of between 900 and 1200 beam interruptions. Within 1 week of the first vehicle or GDNF (10, 100, 500 μg) administration, there was a significant reduction in the locomotor activity of the vehicle injected animals ($P < 0.05$), whereas GDNF injected animals displayed no signs of decline in their locomotor activity (Fig. 3). A two-way ANOVA suggested a highly significant effect of GDNF-treatment ($P < 0.001$). By the second to the fourth week following GDNF injection, there were

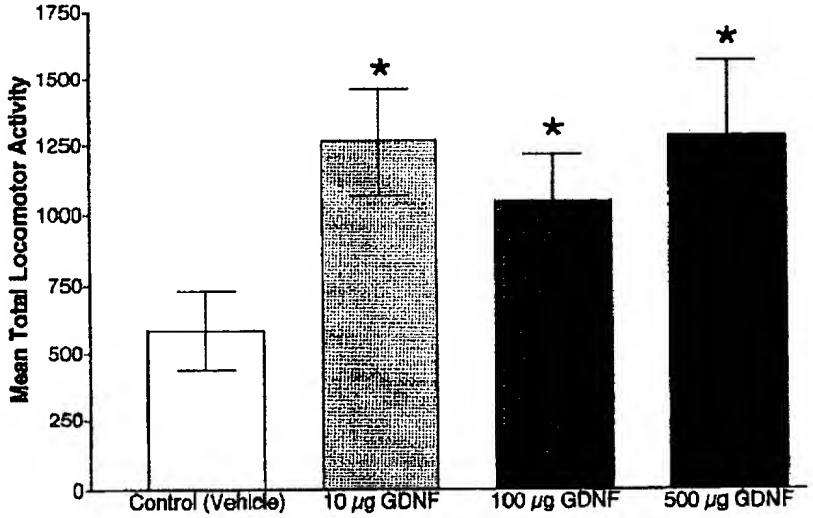


Fig. 4. Mean total locomotor activity for animals receiving vehicle or 10 to 500 μg GDNF. Each histogram represents pooled data for mean locomotor activity of the animals following two administrations of vehicle or GDNF during the entire duration of the experiment. Error bars represent: ± S.E.M. ($n = 4$) * $P < 0.05$, ANOVA followed by Dunnett's test.

marked time dependent improvements in locomotor counts of all animals treated with GDNF compared to the vehicle group. Interestingly, following the second injection of 10 μg GDNF, a dose that had no effect on motor disability, produced a statistically significant ($P < 0.05$) increase in locomotor activity (Fig. 4).

## 4. Discussion

Considerable effort has been devoted to the search for molecules that might exert trophic influences on midbrain dopamine neurones, and have therapeutic value in the treatment of the cardinal symptoms of Parkinson's disease, such as bradykinesia and motor disability. At the start of the experiment, all animal groups had a similar mean activity counts and disability scores, indicating equivalent degree of nigral degeneration induced by MPTP. The data presented in this study demonstrate the effects of two intraventricular injection of GDNF 4 weeks apart on bradykinesia and motor disability. Approximately 3 weeks after the first administration, GDNF dose dependently improved motor disability, characterised by a reduction of motor disability observer scores and improved bradykinesia, as demonstrated by an increased mean locomotor activity over a period of 6 h. We found that only at doses of 100 μg or greater, did GDNF begin to have a positive effect in reducing motor disability. When we tested the higher dose (500 μg), there was a robust and statistically significant improvement in motor disability but some animals in this higher dose group displayed behavioural abnormalities. One type of behavioural abnormality was "obstinate progression". This is a stereotyped behaviour in which animals climb or burrow in their cages despite the presence of barriers (e.g. cage floor or ceiling). In fact one animal died within 2 weeks of the second GDNF administration as a result of injuries incurred during recurring episodes of this behavioural abnormality. At present, it is not certain whether the occurrence of this behavioural abnormality is directly linked with administration of higher doses of GDNF. This behaviour can occur after MPTP treatment alone (see Pearce et al., 1996). Following the second administration of GDNF, improvements in motor disability by 100 and 500 μg GDNF were maintained, but were not further improved.

In general, our findings are in broad agreement with findings in Parkinsonian rhesus monkeys, where significant behavioural improvements were observed in animals receiving 10 to 1000 μg GDNF by the intraventricular route (Zhang et al., 1997). In that study, rhesus monkeys received four doses of GDNF at 4-week intervals and were monitored for an additional 4 months after the last administration. GDNF treatment improved bradykinesia, rigidity, posture and balance for up to 1 month after the last administration (Zhang et al., 1997). Interestingly, these authors also noted that at a dose of 1000 μg, one animal displayed dyskinetic movements. Another major side effect of GDNF administration is an acute weight loss (Zhang et al., 1997; Miyoshi et al., 1997). In the present study, we did not investigate this in detail, however, in a preliminary study reported elsewhere, we did observe weight loss in common marmosets similar to that seen in rhesus monkeys (Iravani et al., 1999).

The observer rating scale used in the studies of rhesus monkey (Gash et al., 1996; Miyoshi et al., 1997; Zhang et al., 1997) is based on a non-human "large" primate Parkinsonian-rating scale (Ovadia et al., 1995). Although our rating scale shares some of the characteristics of the larger primate scale such as posture and balance, but because of characteristics peculiar to common marmoset, our observer rating system differs significantly from that scale. Our observer rating scale includes such features as vocalisation, fur condition (an indication of grooming activity), alertness and head checking (an activity in the normal behavioural repertoire of common marmosets). Therefore, any extrapolations of our data based on those obtained in the rhesus monkey should be treated with some degree of caution.

In order to study the effects of GDNF on bradykinesia, we quantitatively tested the effects of GDNF administration on the locomotor activity in common marmosets. Using locomotor activity cages, the number of times each infrared beam in the three-dimensional array was interrupted, was counted and averaged for a period of 6 h. Injection of saline in control group was initially co-incidental with a decline in the locomotor activity. Although the reason for this detrimental effect is not clear, this effect was not observed in GDNF treated animals. This suggests that GDNF counteracts or reverses the negative effects of i.c.v. saline administration. The overall results show that GDNF even at the low dose used (10 μg), produced a significant enhancement of the locomotor activity. At 100 and 500 μg, GDNF failed to improve the locomotor activity further. The absence of dose effects suggests that peak effects of GDNF on locomotor activity is brought about by much lower concentrations than are necessary to improve motor disability. Furthermore, this finding suggests that improvements in locomotor activity provided by GDNF may come about by a different mechanism from that involved in the amelioration of motor disability.

The potential therapeutic effects of GDNF are thought to be the result of the restoration of dopaminergic neurones. In rhesus monkeys, adult midbrain dopamine neurones stimulated by GDNF showed increased cell size and neuritic extension (Gash et al., 1996). This would explain the long periods required for the expression of the beneficial effects of GDNF on motor disability.

In vivo, GDNF protects dopaminergic neurones from programmed cell death associated with development (Clarkson et al., 1997) and death induced by neuronal transection (Beck et al., 1995). These experiments suggest that GDNF may provide significant therapeutic opportuni-

ties in several neurodegenerative disorders, including Parkinson's disease.

A large body of evidence supports the notion that neurones require trophic support not only during a limited period of ontogenesis, but during their whole life span (see Unsicker, 1994). GDNF may promote survival, transmitter synthesis, and other differentiated properties, and may be crucially important when a neurone is metabolically or toxically impaired. Therefore, it can be argued that a defect in synthesis and/or release of GDNF in a pathological condition may have severe implications for the pathogenesis of Parkinson's disease. For example, there is increasing evidence to suggest that oxidative stress is an important factor in causing Parkinson's disease (Jenner and Olanow, 1998). Recently, it has been shown that administration of recombinant human GDNF resulted in significant increase of glutathione peroxidase, superoxide dismutase, and catalase activities, suggesting that one of the mechanisms of action of GDNF is to protect dopamine neurones through its activation of the antioxidant enzyme systems (Chao and Lee, 1999).

In conclusion, GDNF has been shown to promote the recovery of the injured nigrostriatal dopamine system and improves motor functions in non-human primate models of Parkinson's disease. In the present study, GDNF improves both locomotor activity and disability in MPTP-treated common marmosets. Motoric improvement may reflect a direct action of GDNF on substantia nigra dopamine neurones but an indirect action of GDNF possibly on the activity of the basal ganglia circuitry cannot be ruled out.

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